

Cytoplasmic RNA isolation and immunoprecipitation of dsRNA

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An abbreviated version of this protocol was published in Science Translational Medicine in Jul 2021
Genome-encoded cytoplasmic double-stranded RNAs found in C9ORF72 ALS-FTD brain propagate neuronal loss
DOI: 10.1126/scitranslmed.aaz4699

Detailed protocol

Please find below the protocol for Cytoplasmic RNA isolation and immunoprecipitation of dsRNA below:

Cytoplasmic RNA was extracted from mouse olfactory epithelium/mouse olfactory bulb/50mg specified region of postmortem human brain using RNeasy kit (Qiagen) except that the standard lysis buffer was exchanged for the lysis buffer RNL [50 mM Tris-HCl, pH 8.0; 140 mM NaCl; 1.5 mM MgCl₂; 0.5% (v/v) Nonidet P-40 (1.06 g/ml); and 1 mM DTT added just before use]. Purified RNA was immunoprecipitated using a dsRNA-specific antibody (J2, Scicons) in 200 µl binding buffer (0.025% Triton X-100 in PBS). with 5 µg of J2 antibody and 1 µl RNaseOUT (Life Technologies) rotating overnight at 4°C. J2- bound dsRNA was incubated in binding buffer with 50 µL of prewashed protein-A or G Dynabeads beads for overnight at 4°C, (Life Technologies) followed by 5× washes in cold binding buffer. RNA was then extracted with TRIzol Reagent as above. cDNA was synthesized with SuperScript™ III Reverse Transcriptase (Thermo Fisher).

I hope it helps,

How to cite: (Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Sahin, A. and Albers, M. (2021). Cytoplasmic RNA isolation and immunoprecipitation of dsRNA. Bio-protocol Preprint. bio-protocol.org/prep1328.
2. Rodriguez, S., Sahin, A., Schrank, B. R., Al-Lawati, H., Costantino, I., Benz, E., Fard, D., Albers, A. D., Cao, L., Gomez, A. C., Evans, K., Ratti, E., Cudkowicz, M., Frosch, M. P., Talkowski, M., Sorger, P. K., Hyman, B. T. and Albers, M. W. (2021). Genome-encoded cytoplasmic double-stranded RNAs found in C9ORF72 ALS-FTD brain propagate neuronal loss . Science Translational Medicine 13(601). DOI: [10.1126/scitranslmed.aaz4699](https://doi.org/10.1126/scitranslmed.aaz4699)

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